

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 04 APR 2005

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Applicant's or agent's file reference 300400RU	<b>FOR FURTHER ACTION</b>		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/RU 2003/000474	International filing date (day/month/year) 05 November 2003 (05.11.2003)	Priority date (day/month/year) 12 November 2002 (12.11.2002)	
International Patent Classification (IPC) or national classification and IPC C12N 15/11, 15/10, 15/63, 15/82, A01K 67/00, A01H 1/00, C07K 14/435, 16/18, C12Q 1/68, G01N 33/533			
Applicant ZAKRYTOE AKTSIONERNOE OBSHESTVO "EVROGEN" et al			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This Report consists of a total of 4 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under PCT).

These annexes consist of a total of 7 sheet

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand: 19 May 2004 (19.05.2004)	Date of completion of this report: 03 March 2005 (03.03.2005)
Name and mailing address of the IPEA/ RU FIPS  Russia, 121858, Moskva, Berezhkovskaya nab., 30-1 Facsimile No.	Authorized officer  M. Kuptsova  Telephone No 240-25-91

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International Application No.  
PCT/RU 2003/000474

## I. Basis of the report

## 1. With regard to the elements of the international application:\*

☐ the international application as originally filed☒ the description:

pages 1-26, 28-31, as originally filed  
pages 27, filed with the demand  
pages, filed with the letter of

☒ the claims:

pages, as originally filed  
pages, as amended (together with statement) under Article 19  
pages, filed with the demand  
pages ? 31-33, filed with the letter of 21.12.2004

☒ the drawings:

pages 1/20-20/20, as originally filed  
pages, filed with the demand  
pages, filed with the letter of

☒ the sequence listing part of the description:

pages 1, 5-17, as originally filed  
pages 2, 3, 4, filed with the demand  
pages, filed with the letter of

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1.(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.  
☒ filed together with international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages \_\_\_\_\_  
☐ the claims, Nos. \_\_\_\_\_  
☐ the drawings, sheets/fig. \_\_\_\_\_

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

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## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Claims	1-24	YES
	Claims		NO
Inventive Step (IS)	Claims	1-24	YES
	Claims		NO
Industrial Applicability (IA)	Claims	1-24	YES
	Claims		NO

### 2. Citations and explanation:

The examination report is established for amended claims in view of the following documents:

- D1 – 1GFL A. Chain A, Structure of Green Fluorescent Protein
- D2 – JP 10-234382;
- D3 – US 6232107;
- D4 – US 5976796;
- D5 – WO 1997/041228;
- D6 – CA 2331882.

D1 discloses a primary structure of green fluorescent protein isolated from an organism belonging to the class Hydrozoa having an amino acid sequence, which is about 50% identical to SEQ ID NO:2 characterizing the nucleic acid according to claim 1.

D2 discloses information about a nucleic acid sequence encoding green fluorescent protein, a vector comprising thereof, host cells capable of synthesizing the indicated fluorescent protein. D2 also provides possibility for the use of the obtained protein as a labeling agent for detecting the protein localization in live cells, as a reporter for the analyses of promoters, etc.

D3 discloses primers and probes capable of hybridizing with nucleic acid sequence encoding green fluorescent protein having the length of 14 n.

D4 discloses a green fluorescent and luciferase fusion protein. D4 also discloses a method of making monoclonal antibodies to said protein, a method of making the protein and the possibility of its use as a double marker for monitoring gene expression in living cells and quantitatively by enzymatic activity.

D5 discloses a plant comprising a nucleic acid molecule encoding green fluorescent protein included in expression vector.

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## Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

D6 discloses a mouse comprising an expression system including a nucleic acid encoding green fluorescent protein.

The Applicant has restricted claims 1 and 14 by indicating the minimum possible length of the protein fragment comprising 15 amino acid residues.

Although an amino acid sequence of the fluorescent protein disclosed in D1 is about 50% identical to the sequence shown in SEQ ID NO:2, it does not comprise fragments having 15 amino acid residues and more, which completely coincide in structure with the claimed protein fragments. Hence, features of claims 1 and 14 are not known from D1 and D2-D6.

Consequently, claims 1-4 and 14 meet the criterion of novelty.

The presence of fluorescent proteins in medusas of the genus *Aequorea* gives grounds for search of similar proteins in organisms belonging to other genera but related to the same class Hydrozoa. However, none of the retrieverior art documents teaches that fluorescent proteins of medusas of different genera can have the homology attaining 50%, which makes it possible to use the nucleic acid encoding green fluorescent protein as a tool for the isolation of DNA encoding proteins with similar properties from organisms belonging to Anthomedusae. Hence, an isolated nucleic acid according to claims 1-4 and a protein according to claim 14 and the use thereof for labeling molecules, cells, etc. is not obvious and requires an inventive activity. Based on the foregoing claims 1-4 and 14 meet the criterion of inventive step.

Claims 5-13, 15-24 also meet the criteria of novelty and inventive step, since they contain features of claims 1 or 14.

Claims 1-24 meet the criterion of industrial applicable.